AG Contract No. KR95-2654-TRN
ADOT ECS File No. JPA 95-207
Project: 089A YV 355 H2948 01D
Section: US-89A Arizona Cliffrose
Horticultural Plant Research

AGREEMENT
BETWEEN
THE STATE OF ARIZONA
AND
TRANSITION ZONE HORTICULTURAL INSTITUTE, INC.
aka "THE ARBORETUM AT FLAGSTAFF"

THIS AGREEMENT is entered into _____, 1995, between the STATE OF ARIZONA, acting by and through its DEPARTMENT OF TRANSPORTATION (the "State") and the TRANSITION ZONE HORTICULTURAL INSTITUTE, INC., also known as THE ARBORETUM AT FLAGSTAFF, acting by and through its BOARD OF DIRECTORS (the "Arboretum").

I. RECITALS

1. The State is empowered by Arizona Revised Statutes Section 28-108 to enter into this agreement and has by resolution, a copy of which is attached hereto and made a part hereof, resolved to enter into this agreement and has delegated to the undersigned the authority to execute this agreement on behalf of the State.

2. The Abrboretum is empowered to enter into this agreement and has by Board resolution, a copy of which is attached hereto and made a part hereof, resolved to enter into this agreement and has authorized the undersigned to execute this agreement on behalf of the Arboretum.

3. Incident to roadway reconstruction and highway widening on US-89A contemplated by the State, the new roadway alignment will traverse land under the control and jurisdiction of the US Fish and Wildlife Service (USFWS). The endangered plant species Purshia subintegra, ("the Arizona Cliffrose") is present in the new alignment, and the necessary USFWS permit requires research and mitigation efforts on the plant species by the State. The Arboretum has agreed to provide the required effort on behalf of the State, at an estimated cost of $458,832.00, hereinafter referred to as the Project.

THEREFORE, in consideration of the mutual agreements expressed herein, it is agreed as follows:

II. SCOPE OF WORK

1. The Arboretum will:

a. Establish and conduct a long term (5 to 6 year) research program on the plant species Purshia subintegra. Strictly accomplish the objectives outlined in Exhibit A, which is attached hereto and made a part hereof. Comply will all applicable federal, state and local laws, rules and regulations.

b. Coordinate as appropriate with the USFWS, Coconino National Forest, the National Seed Storage Laboratory, and the State.

c. No more often than monthly, invoice the State for the reasonable direct actual cost of the services provided, in a total amount estimated at $458,832.00, accompanied by narrative reports. Provide the required match in funding or in-kind services in the amount of $27,734.00. Upon completion of the Project, provide a final report. Incorporate State review comments.

2. The State will:

a. Cooperate with the Arboretum in achieving the objectives of the Project. Review narrative and final reports and provide comments. Retain the right to approve any subcontractors or consultants who may be associated with the Project.

b. Reimburse the Arboretum within thirty (30) days after receipt and approval of invoices, in a total amount estimated at $458,832.00.

III. MISCELLANEOUS PROVISIONS

1. This agreement shall remain in force and effect until completion of said Project and reimbursements; provided, however, that this agreement may be cancelled at any time prior to commencement of performance, upon thirty (30) days written notice to the other party. Should the work contemplated under this agreement be completed at a lower cost than the reimbursed amount, or disallowed after audit, or for any other reason should any of these funds not be expended, a proportionate amount of the funds provided shall be reimbursed to the State.

2. This agreement shall become effective upon execution by the parties hereto.

3. This agreement may be cancelled in accordance with Arizona Revised Statutes Section 38-511 as regards conflicts of interest on behalf of state employees.

4. The provisions of Arizona Revised Statutes Section 35-214 pertaining to audit are applicable to this contract.

5. In the event of any controversy which may arise out of this agreement, the parties hereto agree to abide by required arbitration as is set forth in Arizona Revised Statutes Section 12-1518.

6. All notices or demands upon any party to this agreement shall be in writing and shall be delivered in person or sent by mail addressed as follows:

Arizona Department of Transportation
Joint Project Administration
205 South 17 Avenue, Mail Drop 616E
Phoenix, AZ 85007

The Arboretum at Flagstaff
Curator of Plants
PO Box 670
Flagstaff, AZ 86002

IN WITNESS WHEREOF, the parties have executed this agreement the day and year first above written.

TRANSITION ZONE HORTICULTURAL INSTITUTE, INC.

By_____
MICHAEL FOX
Director

STATE OF ARIZONA
Department of Transportation

By_____
A. WAYNE COLLINS
Deputy State Engineer

ATTEST

By_____

Secretary

635
28nov

SCOPE OF WORK

on *Purshia subintegra*

Submitted by:
Joyce Maschinski, Curator of Plants
The Arboretum at Flagstaff
November 3, 1995

Purpose: The proposed extensive research program is designed to contribute to the knowledge of the biology of *Purshia subintegra* in its natural environment and in a captive *ex situ* setting for purposes of conservation of the species. The captive *ex situ* population will be maintained as a safeguard against demise of wild populations and as an "experimental" subgroup to learn about cultivation requirements and environmental restrictions of the species. Wild populations will be studied to determine *in situ* viability and general ecology of the species.

OBJECTIVES OF RESEARCH:

1) To develop a genetically representative *ex situ* seed bank of *Purshia subintegra* from the Verde Valley.

2) To determine cultivation requirements for seeds, cuttings, and transplanted individuals.

3) To determine long-term population viability of the species through establishing demographic studies of the Verde Valley population.

4) To determine the ecological requirements for seedling establishment and survival.

5) To determine the extent to which endemic soil attributes, including mycorrhizae, water availability, and nutrients may restrict expansion of *Purshia subintegra* into new habitats.

6) To determine the extent to which the morphological characters of *Purshia subintegra* and its hybrids with *Purshia stansburiana* persist across nutrient and soil gradients.

PROPOSED METHODOLOGY FOR MEETING OBJECTIVES

OBJECTIVE 1. To develop a genetically representative *ex situ* seed bank of *Purshia subintegra*.

Seed banks are standard practice recommended to conserve the genetic diversity of a species and safeguard it against demise of the wild population. The Center for Plant Conservation recommends that seeds from at least 100 individuals in at least five populations be obtained so that the diverse genetic composition of a species is represented.

In order to achieve this recommendation for the Verde Valley population of Purshia subintegra, it will be necessary to collect seed from the Verde Valley over several years. Collections will be necessary over several years, because there is variation and limitations on the number of seeds produced in a year and restrictions on the allowable number of seeds that can be collected under permit with federal agencies and landowners. The seed collection will include seeds from individuals currently growing in the Hwy 89A right-of-way, if seeds are available.

Collected seed will be used for three purposes. A third of the seed will be shipped to the National Seed Storage Laboratory in Ft Collins, Colorado for long-term storage. The remaining two-thirds of the seed will be kept at The Arboretum at Flagstaff: one-third will be placed in in-house storage and one-third will be used for germination studies.

OBJECTIVE 2. To determine cultivation requirements for seeds, cuttings, and transplanted individuals.

Central to the conservation of Purshia subintegra is developing an understanding of its cultivation requirements. With this knowledge, it will enable us to augment declining or threatened populations, to conduct experiments on ecological requirements of the species, and to germinate seeds from in-house or long-term storage should it become necessary.

SEEDS

According to germination studies done with the common cliffrose, Purshia stansburiana, germination at a success rate of 55% occurred after 2 weeks of stratification at 5 C, followed by night day temperatures at 10/20, 10/25, 10/30 and 15/25 C (Young and Young 1992). Previous germination tests done on Purshia subintegra at The Arboretum at Flagstaff yielded only 7% germination after 2.5 months of stratification and incubation at 15/20 C. To increase the probability of germination, we propose to use germination treatments following the above guidelines for Purshia stansburiana. In addition, the effects of fire on seed germination will be assessed in a separate treatment. Seedlings will be used for experiments described for objectives 4 - 6.

CUTTINGS

In order to preserve the genotypes that are currently growing in the Hwy 89A right-of-way, cuttings will be taken from each individual. In addition, cuttings will be taken from representative populations in the Verde valley so that rooted cuttings can be used for experiments described for objectives 4 & 5. Because previous propagation trials with cuttings had low success, (i.e., 2% of 144 cuttings successfully rooted and survived), we propose to take cuttings systematically over the growing season to determine the optimal time to take cuttings for greatest success. When possible, we will age the wood being used to propagate cuttings to determine if age influences rooting success. We will also manipulate misting frequency and propagation media to increase rooting success.

TRANSPLANTED INDIVIDUALS

Plants generated from cuttings or seeds that are not needed for experiments will be transplanted into varying microsites on the grounds of The Arboretum at Flagstaff and in the Verde Valley to determine the method and microsite that will be optimal for transplanting success. The microsite conditions created before transplanting will be defined, in part, by the results of experiments in objective 4.

OBJECTIVE 3. To determine long-term population viability of the species through establishing demographic studies.

Demographic information is one of the most valuable data sets that can be gathered on a species, because it can be used to assess population trends over time, the species risk of extinction, the most vulnerable life history stages, and the associated environmental factors that may favor population growth.

In the Verde Valley, we propose to establish 10 belt transects to assess plant size, stage, survival/mortality, flowering, seed production, and seedling establishment. If attempts to age the individuals are successful, then age will also be measured for individuals within the transects. Demographic data will be gathered over the next 5 + years. Data will be analyzed using population viability analysis.

In addition, microsite data will be gathered on the transects to determine if there is a relationship between population growth and environmental factors (as described in objective 5).

OBJECTIVE 4. What ecological factors are correlated with seedling establishment and survival?

In the Verde Valley, there are several areas where seedlings have become established. We propose to map and monitor the survival of seedlings over 4 years. At each site, we will measure ecological attributes, including slope, aspect, nearest neighbor, soil composition, and light and compare these to randomly selected sites where adult Purshia is present, but seedlings are absent.

Further, we propose to do on site germination tests and seedling survival tests, using seed collected from nearby adults and seedlings produced from objective 2. We propose to establish seed and seedlings in the Verde Valley into each of 10 replicated plots within 4 different microsites. Microsite attributes will be based on the findings from the ecological attribute study.

OBJECTIVE 5. Are endemic soil conditions restricting the expansion of Purshia subintegra into new habitats?

Understanding factors that restrict the expansion of rare species is central to the conservation of the species. The current biogeographic distribution of Purshia subintegra led Anderson (1993) to conclude that the species is able to grow on a wide range of soil types, but is restricted to lacustrine nutrient poor sites due to competition with creosote. However, cultivation trials at The

Arboretum at Flagstaff suggest that the species is obligated to grow in native, unsterilized nutrient poor soils, because rooted cuttings died if they were transplanted into non-native nutrient rich soils. Any future modifications of Purshia subintegra habitat may need to consider microhabitat requirements of the species in order to safeguard populations. Similarly, knowing microhabitat requirements may allow augmentation of threatened or declining populations.

Several factors may be involved in a species obligatory growth on a particular soil. These include mycorrhizal communities, nutrient and/or water availability. Mycorrhizal symbionts are important for aiding nutrient uptake particularly in soils containing relatively low amounts of available nitrogen and phosporus (i.e., Hacskaylo and Snow 1959). The distributions of several species are determined by the presence or absence of mycorrhizae (Vozzo and Hacskaylo 1971, Berliner et al. 1986).

Nutrient conditions may also limit Purshia subintegra growth to a particular soil formation. Anderson (1993) found that soils where Purshia subintegra was present had significantly lower amounts of organic matter and phosphorus, yet higher amounts of magnesium and potassium than soils where Purshia subintegra was absent. No information has been gathered on soil chemistry in hybrid zones versus pure zones of Purshia subintegra.

The following experiments are proposed to examine the factors restricting Purshia subintegra growth to nutrient poor lacustrine soils.

MYCORRHIZAL EFFECTS

Before inititating experiments on mycorrhizae, roots of plants in the 89A right-of-way will be excavated and examined for presence of ectomycorhizae and endomycorrhizae by staining with trypan blue following methods described in Berlinger et al. (1986) and Gehring and Whitham (1991). Intensity of mycorrhizal infection will be quantified.

Experiment 1. To determine whether endemic mycorrhizal communities are required for Purshia subintegra growth, we propose to compare growth and survival on soils where pure Purshia subintegra grows (PS), soils where hybrids occur (HS), and soils where Purshia stansburiana occurs (PST). Seedlings will be transplanted into unsterilized and sterilized PS, HS, and PST soils inoculated with sterile plugs, native soil plugs with mycorrhizae present, or sterilized soil inoculated with commercially available vesicular-arbuscular mycorrhizae. Each treatment will be replicated 30 times in each of the 3 parental soils for a total of 450 plants. Plants will be maintained in 2 gallon pots in a heated greenhouse facility at the Arboretum at Flagstaff and each pot will be placed on a drip irrigation system so that all plants will receive equal amounts of water.

To establish growth rates in the different soils, plant height and number of leaves will be measured every three - five weeks for the first year and every 8 weeks in the second year.

After 6 months, a subsample of 10 plants in each group will be destructively sampled for shoot biomass, root biomass, and mycorrhizal infection using methods described in Berlinger et al. (1986) and Gehring and Whitham (1991).

NUTRIENT AVAILABILITY

Experiment 2. To determine whether changes in nutrient conditions will affect growth and development of Purshia subintegra and composition of associated plants in its habitat, we propose to examine plant nutrient uptake in experimental and control plots. Plants, such as Purshia subintegra, which are adapted to low nutrient conditions may lack the ability to increase growth rates when nutrient levels are increased (Grime 1979). By manipulating nutrients in the soils, it will be possible to determine whether 1) Purshia subintegra can increase growth rates when nutrient levels are increased; 2) whether supplemented nutrient conditions in Purshia subintegra habitats will change the composition of the plant community or mycorrhizal community, and 3) whether changes in the composition of the plant community will adversely or beneficially affect Purshia subintegra.

To determine what nutrients are present in the soil, soil samples will be taken at 75 randomly selected study plots. Study plots will encompass a single plant and a 2 m radial circle around the central plant. For two years, a random subsample of 25 of the plots will be supplemented with phosphorus in dry powdered form in the winter and summer. (Phosphorus is the nutrient Anderson (1993) identified as being deficient in Purshia subintegra habitat.) Twenty-five more plots will be supplemented with nitrogen. Methods will follow Huenneke et al. (1990). If plots are exposed to grazing, fencing will be required.

Plant height, number of branches, number of flowers, and seed set for all plants within study plots will be measured before and after each nutrient treatment. The number and identities of other plant species in the experimental and control plots will also be counted. Root samples will be excavated to measure intensity of mycorrhizal infection.

Concentrations of magnesium, potassium, phosphorus, manganese, iron, and nitrogen will be measured and compared in soil and 10 grams of leaf tissues collected before the first nutrient application and 3 months after each nutrient application. The effect of nutrients on plant growth and reproduction will be analyzed using canonical correlation analysis.

Soil nutrients from experimental plots will be compared to soil nutrients collected from 10 sites thought to have potential for reintroduction of Purshia subintegra. These data will be analyzed using canonical correlation analysis.

OBJECTIVE 5. To determine the extent to which the morphological characters of Purshia subintegra and its hybrids with Purshia stansburiana persist across nutrient and soil gradients.

Experiment 3. Stem cuttings from pure stands of P. subintegra, P. stansburiana, and hybrids will be collected, propagated, and transplanted into 2 gallon pots containing one of each of the three parent soils (collected from the same cutting collection sites). All soil will be unsterilized, therefore periodic weeding will be necessary. Twenty replicates of each species will be placed into one of the three soil types for a total of 180 plants in 180 pots. Potted

plants will be maintained in a heated greenhouse facility at The Arboretum at Flagstaff. Each pot will be placed on a drip irrigation system so that all plants will receive equal amounts of water.

Leaf and flower morphometric characters, presence or absence of glands on leaves and hypanthia will be measured after 6, 12 and 18 months. These data will be analyzed using a discriminant function analysis.

LITERATURE CITED:

Anderson, J.L. 1993. A synthetic analysis of a rare Arizona species, Purshia subintegra (Rosaceae). Pages 205-220 in B. Sivinski and K. Lightfoot, editors. Proceedings of the Southwestern Rare and Endangered Plant Conference. New Mexico Forestry and Resources Conservation, No. 2.

Berliner, R., B. Jacoby, and E. Zamski. 1986. Absence of Cistus incanus from basaltic soils in Israel: effect of mycorrhizae. Ecology 67: 1283-1288.

Gehring, C.A. and T.G. Whitham. 1991. Herbivore-driven mycorrhizal mutualism in insect-susceptible pinyon pine. Nature 353:556-557.

Grime, J.P. 1979. Plant strategies and vegetation processes. John Wiley & Sons, New York, New York.

Hacskaylo, E. and A.G. Snow. 1959. Relation of soil nutrients and light to prevalence of mycorrhizae. USDA Sta. Paper No. 125, North Eastern For. Serv. 13 p.

Huenneke, L.F.,S.P. Hamburg, R. Koide, H.A. Mooney, and P.M. Vitousek. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71:478-491.

Koide, R., L.F. Huenneke, and H.A. Mooney. 1987. Gopher mound soil reduces growth and affects ion uptake of two annual grassland species. Oecologia 72:284-290.

Vozzo, J. A., and E. Hacskaylo. 1971. Inoculation of Pinus caribaea with ectomycorrhizal fungi in Puerto Rico. Forest Science 17:239-245.

Yoide, J.A. and C.G. Young. 1992. Seeds of Woody Plants in North America. Dioscorides Press. Portland, Oregon.

RESEARCH SCHEDULE

YEAR 1996

* Build greenhouse facility for propagation.
* Install growth chamber.
* Collect seed and cuttings; Begin propagation.
* Establish demographic transects.
* Excavate roots 89A plants to examine mycorrhizae.
* Select plots for Nutrient Study. (Initiate Nov 1996 - End Nov 1998)
  Soil and Leaf Nutrient Analysis.
  Apply fertilizer to Nutrient Plots.

YEAR 1997

* Collect cuttings; Cuttings propagation.
* Collect Seed.
* Initiate Seed Germination Studies.
* Monitor Demographic transects.
* Initiate Mycorrhizal studies in greenhouse.
  (Initiate June 1997 - End June 1999)
  Do measurements on plants in Mycorrhizal studies.
* Measure plants in Nutrient Study.
  Soil and Leaf Nutrient Analysis.
  Apply fertilizer to Nutrient Plots.

YEAR 1998

* Do measurements on plants in Mycorrhizal studies.
  Sample Roots in mycorrhizal studies for mycorrhizal infestation.
* Continue Germination Studies.
* Monitor Demographic transects.
* Measure plants in Nutrient Study.
  Soil and Leaf Nutrient Analysis.
  Apply fertilizer to Nutrient Plots.
* Seed collection.

YEAR 1999

* Do measurements on plants in Mycorrhizal studies.
* Analyze nutrient data; write report and paper.
* Monitor Demographic transects.
* Begin Propagation for Morphological studies and Initiate studies.
  Make Measurements on plants in Morphological studies.
* Conduct Transplant studies.

YEAR 2000

* Analyze Mycorrhizal studies; write report and paper.
* Continue Analysis Nutrient studies; write report and paper.
* Monitor Demographic transects.
* Make Measurements on plants in Morphological studies.
* Monitor Transplant studies.

YEAR 2001

* Continue writing up Mycorrhizal studies.
* Continue writing up Nutrient studies.
* Monitor Demographic transects.
  Analyze Demographic Data.
  Write up report and paper.
* Make Measurements on plants in Morphological studies.
  Analyze Morphological Data; write up report and paper.
* Monitor Transplant studies.

JPA 95-207

RESOLUTION

BE IT RESOLVED on this 28th day of November 1995, that I, the undersigned LARRY S. BONINE, as Director of the Arizona Department of Transportation, have determined that it is in the best interests of the State of Arizona that the Department of Transportation, acting by and through the Highways Division, to enter into an agreement with the Arboretum at Flagstaff for the purpose of defining responsibilities to conduct a long term research project on the endangered plant species Arizona Cliffrose incident to a reconstruction and widening project on US-89A.

Therefore, authorization is hereby granted to draft said agreements which, upon completion, shall be submitted to the Deputy State Engineer for approval and execution.

for LARRY S. BONINE
Director

ATTORNEY GENERAL
CIVIL DIVISION
TRANSPORTATION SECTION

MEMORANDUM

TO: E. JACK HAMMITT 616E
Joint Project Administrator

FROM: JAMES R. REDPATH
Assistant Attorney General

DATE: January 29, 1996

RE: CON/Transition Zone Horticultural Institute, Inc.
aka "The Arboretum at Flagstaff"
A.G. Contract No.: KR95-2654-TRN
ECS File: JPA 95-207
Project: 089A YV 355 H2948 01D
Section: US-89A Arizona Cliffrose
Horticultural Plant Research

This agreement appears to be in proper form and may be circulated for signature.

JAMES R. REDPATH
Assistant Attorney General

JRR:lsr
9042G/17